Aquaculture Research, 2007, **38,** 1747–1758 doi: 10.1111/j.1365-2109.2007.01854.x

Effect of protein source and nutrient density on growth efficiency, histology and plasma amino acid concentration of rainbow trout (*Oncorhynchus mykiss* Walbaum)

F T Barrows[1], T G Gaylord[1], D A J Stone[2] & C E Smith[3]

[1]U.S. Department of Agriculture, Agricultural Research Service, Small Grains and Potato Germplasm Research Unit, Hagerman Fish Culture Experiment Station, Hagerman, ID, USA

[2]University of Idaho, Hagerman Fish Culture Experiment Station, Hagerman, ID, USA

[3]Smith Pathology and Aquaculture Consulting, Bozeman, MT, USA

Correspondence: F T Barrows, USDA/ARS/PWA Hagerman Fish Culture Experiment Station, 3059-F National Fish Hatchery Road, Hagerman, ID 83332, USA. E-mail: Rick.Barrows@ars.usda.gov

Abstract

The effects of protein source and nutrient density on growth efficiency, nutrient digestibility and plasma amino acid concentrations of rainbow trout were evaluated. A 3 by 2 factorial treatment design with three protein sources, fish meal–barley (F–B), plant concentrates (PC) and plant meals (PM), and two nutrient densities were used. A commercial reference diet was also fed. Triplicate tanks of 30 fish (initial wt. 28 g) were fed each diet, and the final weight averaged 240 g fish^{-1}. Protein source and nutrient density affected feed intake, weight g ain and feed conversion ratio. Weight gain of trout fed the PC and PM diets was approximately 10% less than fish fed the F–B diets. Protein retention was affected by protein source, but not nutrient density, and was the highest for the fish fed diets containing fish meal and the lowest for the fish fed PM diets. Apparent digestibility coefficients and apparent amino acid availabilities of the diets corresponded with differences in weight gain. This study provides further evidence that growth rates of trout fed fish meal-free diets, using conventional and concentrated plant protein ingredients, are good but some limitation to growth exists in the fish meal-free diets.

Keywords: rainbow trout, alternate protein source, plant-based feeds

Introduction

The search for alternatives to fish meal in rainbow trout diets has been ongoing since the 1970s (Cho, Bayley & Slinger 1974; Dabrowska & Wojno 1977; Higgs, Markert, Macquarrie, McBrie, Dosanjh, Nichols & Hoskins 1978). In the last decade, however, rising fish meal prices, intense regulation of nutrients in hatchery effluents and the debate on the sustainability of fish meal as an aquafeed ingredient have intensified research in this area (Kaushik, Cravedi, Lalles, Sumpter, Fauconneau & Laroche 1995; Adelizi, Rosati, Warner, Wu, Muench, White & Brown 1998; Barrows & Hardy 2001; Lee, Dabrowski, Blom, Bai & Stromberg 2002; Yamamoto, Shima, Furuita & Suzuki 2002). Soybean meal has been studied extensively as a partial replacement for fish meal due to its abundance and relative price (Fowler 1980; Reinitz 1980; Olli, Krogdahl & Berg-Lea 1989; Arnesen, Brattås, Olli & Krogdahl 1990; Krogdahl, Berg-Lea & Olli 1994; Arndt, Hardy, Sugiura & Dong 1999; Refstie, Korsoen, Storebakken, Baeverfjord, Lein & Roem 2000; Vielma, Makinen, Ekholm & Koskela 2000; Refstie, Storebakken, Baeverfjord & Roem 2001). Corn products, including corn gluten meal, corn gluten feed and whole yellow corn, are also readily available and have been used as a partial replacement for fish meal (Ketola & Harland 1993; Adelizi *et al.* 1998; Stone, Hardy, Barrows & Cheng 2005). Terrestrial animal by-product meals such as blood meal, poultry by-product

meal, meat and bone meal and feather meal have been evaluated due to their relatively low cost and abundant supply (Bureau, Harris, Bevan, Simmons, Azevedo & Cho 2000; Erturk & Sevgili 2003; Cheng, Hardy & Huige 2004). Ingredients not commonly seen in US markets, such as faba beans, pea meal and lupin meals, have also been evaluated as protein sources in trout diets (Gomes, Rema & Kaushik 1995; Drew, Racz, Gauthier & Thiessen 2005; Glencross, Evans, Dods, McCafferty, Hawkins, Maas & Sipsas 2005; Glencross, Hawkins, Evans, Rutherford, Dods, Maas, McCafferty & Sipsas 2006).

To improve the nutritive value of plant products for fish, ingredients are being modified by chemical, mechanical and biological methods. Fermentation of plant products can result in improved nutrient profiles by removing non-nutritive or anti-nutritive components (Mukhopadhyay & Ray 1999; Skrede, Storebakken, Skrede, Sahlstrom, Sorensen, Shearer & Slinde 2002; Ng, Lim, Lim & Ibrahim 2002; Bairagi, Gosh, Sen & Ray 2004; Refstie, Sahlstrom, Brathen, Baeverfjord & Krogedal 2005). Chemical and mechanical processing can also remove anti-nutrients and/or fractions of low nutritive value that results in high-protein plant products that are commercially available, but are more expensive than the more common ingredients (Kaushik *et al.* 1995; Adelizi *et al.* 1998; Bureau, Harris & Cho 1998). Air-classification is a mechanical method that uses air pressure and particle density to separate different fractions. High-protein meals can be produced from oats (Wu & Stringfellow 1973) and wheat (Wu & Stringfellow 1992; Letang, Samson, Lasserre, Chaurand & Abecassis 2001). Rice, pea and barley protein concentrates produced using air classification are commercially available (Parhiem Foods, Saskatchewan, Canada).

Feeding high nutrient density (HND) diets has been demonstrated to decrease nutrient excretion (Medale, Brauge, Vallee & Kaushik 1995; Yigit, Yardim & Koshio 2002). Feed conversion ratios (Weatherup, McCracken, Foy, Rice, McKendry, Mairs & Hoey 1997; Rasmussen, Ostenfeld & McLean 2000) and growth were also improved when diets containing high lipid levels were fed to rainbow trout (Encarnacao, de Lange, Rodehutscord, Hoehler, Bureau & Bureau 2004). HND diets contain increased energy and/or protein levels and are being fed in increasing amounts in the US trout industry relative to the standard feeds. Crude protein levels of 45–50% and total fat levels up to 20% are being fed more frequently by US trout producers, especially when trout prices are high. The increased nutrient density of these feeds requires the use of high-protein ingredients, and the reduction or elimination of ingredients with high carbohydrate levels, such as unrefined grains. Plant protein concentrates (corn gluten meal, soy protein concentrate and wheat gluten meal) are valuable to feed formulators when reducing the fish meal content of the diet.

The objective of the current study was to determine the effect of protein source and nutrient density on fish performance, in order to identify a fish meal-free diet composed of commercially available ingredients to use as a benchmark for future improvements in plant-based feeds.

Materials and methods

Experimental design

A 4 by 2 factorial treatment design was used with, protein source and nutrient density as the main effects. Protein sources were fish meal–barley (F–B), plant concentrates (PC) or plant meals (PM) (Table 1). The diets were manufactured with each protein source to contain either 43% protein and 13% fat (low nutrient density, LND) or 48% protein and 18% fat (HND). These combinations result in six experimental diets (Table 1). The protein and lipid levels used in this study were chosen to reflect current commercial trout feed formulations. Crude rather than digestible protein and energy values were used because a complete set of digestibility values using the same methods was not available. A commercial trout feed (Steelhead, 46% protein and 16% fat, Silver Cup Fish Feeds, Murray, UT, USA) was also evaluated as a reference to commercial-type diets.

Plant concentrates were chosen to have high levels of protein and reduced concentrations of anti-nutrients. Rice protein concentrate (Remypro 70, A&B ingredients, Fairfield, NJ, USA) contains 70% and is produced using air classification. Barley protein concentrate (25% protein, Parhiem Foods, Saskatchewan, Canada) was also produced using air classification. The PM diets contained plant-derived protein sources that are readily available in large quantities and included soybean meal, corn gluten meal and wheat gluten meal and wheat flour. Synthetic lysine and methionine were added to diets, as needed, to meet the amino acid requirements (NRC 1993).

Fish and culture

Thirty rainbow trouts (House Creek strain, College of Southern Idaho), with an average initial weight of

Table 1 Ingredient composition (g 100 g^{-1}) of experimental diets

	Fish barley		Plant concentrate		Plant meals	
	Low	High	Low	High	Low	High
Ingredient						
Fish meal*	47.68	59.24	–	–	–	–
Rice Protein, 70%†	–	–	19.72	26.27	–	–
Wheat Gluten meal	–	–	–	–	7.04	8.60
Corn Gluten meal	–	–	–	–	34.57	42.22
Soy protein concentrate	–	–	24.23	32.22		–
Soybean meal	–	–	–	–	18.96	23.14
Barley Protein‡	–	–	29.03	20.66	–	–
Barley meal	32.30	26.24	8.51	–	–	–
Poultry by-product, meal§	–	–	–	–	–	–
Blood meal¶	–	–	–	–	–	–
Wheat flour	9.70	–	–	–	20.96	.61
Fish oil, menhaden	7.30	13.5	11.16	16.26	11.43	16.79
Soy lecithin	2.00	2.00	2.00	2.00	2.00	2.00
Lysine-HCL	–	–	.97	1.03	1.47	1.97
DL-Methionine	–	–	.32	–	–	–
Di-calcium phosphate	–	–	3.19	3.73	2.55	3.65
Vitamin premix¶	0.40	0.40	0.40	0.40	0.40	0.40
Choline Cl	0.50	0.50	0.50	0.50	0.50	0.50
Ascorbic acid	0.02	0.02	0.02	0.02	0.02	0.02
Trace mineral pre.‖	0.10	0.10	0.10	0.10	0.10	0.10
Analysed composition**						
Protein, % as fed	44.8	49.8	44.3	48.9	42.4	47.6
Lipid, % as fed	12.8	18.0	13.6	17.7	13.3	20.0
Moisture, %	7.0	7.2	7.2	7.8	7.6	6.9
Ash, % as fed	4.5	5.5	5.0	5.4	4.3	5.3
Arginine, % dm	3.30	3.96	4.00	4.59	2.35	2.43
Histidine, % dm	1.13	1.37	1.15	1.26	0.99	1.11
Isoleucine, % dm	2.29	2.53	2.06	2.24	1.88	2.10
Leucine, % dm	3.95	4.31	3.68	4.03	5.38	6.39
Lysine, % dm	3.62	4.24	2.87	3.18	2.44	2.83
Methionine, % dm	1.21	1.49	0.88	0.85	0.76	0.89
Phenylalanine, % dm	2.32	2.52	2.55	2.75	2.61	2.95
Threonine, % dm	2.22	2.55	1.71	2.04	1.65	1.82
Tyrosine, % dm	1.86	1.99	2.01	2.31	2.02	2.35
Valine, % dm	2.62	3.04	2.50	2.74	2.11	2.39

*Peruvian anchovy, 70% protein, Silver Cup Fish Feeds, Murray, UT.

†RemyPro, 70% protein, A&B Ingredients, Fairfield, NJ.

‡Barley protein concentrate, 25% protein, Parhiem Foods, Saskatchewan, CA.

§Blood meal, 82% protein, Silver Cup Fish Feeds, Murray, UT.

¶Contributed per kilogram of diet: vitamin A (as retinol palmitate), 10 000 IU; vitamin D_3, 720 IU; vitamin E (as DL-%-tocopheryl-acetate), 530 IU; niacin, 330 mg; calcium pantothenate, 160 mg; riboflavin, 80 mg; thiamin mononitrate, 50 mg; pyridoxine hydrochloride, 45 mg; menadione sodium bisulphate, 25 mg; folacin, 13 mg; biotin, 1 mg; vitamin B_{12}, 30 µg.

‖Contributed in mg kg^{-1} of diet: zinc, 37; manganese, 10; iodine, 5; copper, 3.

**Guaranteed composition of commercial trout feed; 45% protein, 19% lipid, 3% fiber, 12% ash, moisture < 10%.

38 g, were randomly placed in 30, 150-L fibreglass tanks, each supplied with 10 L min^{-1} of untreated, constant temperature (14.5 °C), gravity-fed spring water at the Hagerman Fish Culture Experiment Station, University of Idaho. There were three tanks of fish per diet and fish were fed three times per day, 6 days per week to apparent satiation for a period of 86 days. A 14-h photoperiod, controlled by timers and fluorescent lights, was provided. The experimental protocol was approved by the University of Idaho's Animal Care and Use Committee.

To determine the digestibility of dietary nutrients, the diets were evaluated following the growth trial. Fish from each treatment were pooled and

redistributed into two 500-L tanks, with 20 fish per tank, for a total of 12 tanks. The larger tanks used in the digestibility phase of the project were needed to accommodate the larger fish size, and this required a reduction from three replicates per diet in the growth phase to two replicates per diet in the digestibility phase. The fish were fed their respective diets to apparent satiation twice daily for 5 days. All diets contained 0.1% yttrium oxide as an inert marker. Faeces were collected by hand stripping from all fish within each tank. Faeces were pooled by tank and stored at − 20 °C until analysed. Apparent digestibility coefficients (ADC) were determined for organic matter, lipid, energy, protein and amino acids.

Diet preparation

Before mixing the diets, all ingredients were ground using an air-swept pulverizer (Jacobsen 18 H, Minneapolis, MN, USA). Dry ingredients were mixed in a horizontal mixer and a portion (∼ 1/3) of the added oil was mixed into the dry ingredients along with the lecithin. The mash was then extruded through a 3.0 mm die of a Buhler twin-screw cooking extruder (DNDL-44, Buhler AG, Uzwil, Switzerland). Barrel temperature averaged 127 °C in sections 2–6, and die pressure was ∼ 410 psi and the feed had a barrel residence time of approximately 18 s. The diets were dried in a pulse bed drier extruder (Buhler AG) with the air discharge temperature remaining below 104 °C, and the final moisture content < 8%. After the diets were dried, they were top-coated with the remaining oil (6%) at ambient pressures, and stored at room temperature (∼ 18–23 °C).

Diet analyses

Feed and faecal samples were dried, and analysed using AOAC (1990) methods for proximate composition, with the exception of protein and crude lipid. Dried samples were finely ground using mortar and pestle and analysed for crude protein (total nitrogen × 6.25) using a LECO FP-428 nitrogen analyser (LECO Instruments, St. Joseph, MI, USA). Crude fat was analysed using a soxhlet extraction apparatus (Soxtec System HT, Foss Tecator AB, Hoganas, Sweden) with methylene chloride as the extracting solvent, and ash by incineration at 550 °C in a muffle furnace. The energy content of the samples was determined using a Parr bomb calorimeter (Parr Instrument Co., Moline, IL, USA). Yttrium analyses were conducted at the University of Idaho Analytical Sciences Laboratory, Moscow, ID, USA, using an Optima 3200 radial inductively coupled plasma atomic emission spectrometer (Perkin-Elmer Corp., Norwalk, CT, USA).

Performance indices and apparent digestibility/availability coefficients

The concentration of moisture, crude protein ($N \times 6.25$), essential amino acids, energy in the feed and fish at the beginning and at the end of the study were determined. Crude protein, energy and dry matter were measured in the feed and fish, and amino acids were measured in the feeds also. The amount of each nutrient fed during the study was used to calculate apparent nutrient retention during the 86-day study. Indices were expressed on a per-fish basis for each dietary treatment group. Performance indices were calculated using the following formulae:

Specific growth rate (SGR) = (final weight–initial wt)/duration of experiment (86 days).

Feed intake expressed as a percent of body weight per day was calculated as a percentage of the average of the initial and final weights per fish from each tank.

Feed conversion ratio (FCR) = feed intake (dry weight)/body weight gain (wet weight).

Apparent protein retention efficiency (PRE%) = protein gain in fish (g)/protein intake in feed (g) × 100.

Apparent energy retention efficiency (ERE%) = energy gain in fish (g)/energy intake in feed (g) × 100.

Apparent digestibility (ADC) or availability (AAC) coefficients of diets for organic matter, protein, essential amino acids and energy were calculated using yttrium oxide as the inert marker and the following formula:

Diet ADC or AAC (%) = 100 × [1 − (% Yttrium in diet/% yttrium in faeces) × (% nutrient in faeces/% nutrient in diets)] (Cho & Slinger 1979).

Histology

Fish were sampled at the end of the trial for histological analyses. Five fish from each of the replicate tanks were euthanized and samples of the kidney, liver and pyloric and rectal intestines were preserved in Davidson's solution for 48 h. Tissues were then transferred to 65% alcohol until processed

by standard histological procedures (Sheehan & Hrapchek 1983). Fish from the LND diet series were evaluated first, and because there was a lack of significant dietary effects in that series, the fish from the HND series were not evaluated.

Plasma amino acids

Blood was collected from the caudal vasculature of 5 fish per tank, 6–8 h post prandial using heparinzed syringes at the end of the growth study. The plasma was separated following centrifugation at 1000 × *g* for 10 min. Plasma was stored at − 80 °C until analysis and was pooled by tank. Plasma amino acids were quantified according to Fleming, Taylor, Miller and Woodward (1992) using an Agilent 1100 series HPLC and *o*-phthaldialdehyde pre-column derivatization of amino acids. Before separation, 50 µL of plasma was diluted with 50 µL water. Plasma proteins were then precipitated out with 150 µL of 1.5 M perchloric acid followed using centrifugation at 3000 × *g* for 5 min. Next, 100 µL of the resulting supernatant was prepped for injection with 1.2 mL water, 100 µL 1.2% benzoic acid, and 100 µL saturated potassium tetraborate. After vortexing, the mixture was filtered through a 0.22 µm syringe filter. Samples were derivatized with o-phthaldialdehyde (Sigma-Aldrich Co., St. Louis, MO, USA) immediately before injection on a 5 µm Agilent Hypersil AA ODS column (Agilent Technologies, Palo Alto, CA) using an automated injection sequence.

Statistical analyses

Fish performance, nutrient retention and nutrient digestibility, plasma amino acids and carcass composition data were analysed using the general linear models procedure of the Statistical Analysis System (SAS 1988). Differences in treatment means were separated using Duncan's multiple range test. Any value expressed as a percentage was arcsine transformed before analysis (Sokal & Rohlf 1981). The performance and nutrient retention data were also analysed as a 3 by 2 factorial treatment design.

Results

Fish performance

There was an effect of protein source and nutrient density, but no interaction, on weight gain and feed conversion ratio (Table 2). Weight gain, expressed

Table 2 Effect of diet on feed consumption and growth efficiency of rainbow trout

Source	Fish barley		Plant concentrate		Plant meals			Probability of $a > F$ value				
Density	Low	High	Low	High	Low	High	Commercial trout	SEM	Model	Source	Density	Source × density
Gain, g fish^{-1}	198.6^{abc}	224.0^{ab}	194.3^{bc}	195.7^{b}	183.0^{c}	202.3^{abc}	229.7^{a}	6.76	0.001	0.02	0.01	0.20
SGR, g fish^{-1} d^{-1}*	2.3^{abc}	2.60^{ab}	2.26^{bc}	2.27^{bc}	2.13^{c}	2.35^{abc}	2.67^{a}	0.08	0.001	0.01	0.01	0.12
FCR†	0.93^{cd}	0.84^{d}	1.02^{b}	0.95^{bc}	1.12^{a}	0.98^{bc}	0.88^{cd}	0.02	0.001	0.01	0.01	0.12
Feed intake, g fish^{-1}	183.7^{b}	187.8^{ab}	198.2^{ab}	184.4^{b}	205.4^{a}	197.4^{ab}	203.2	5.80	0.001	0.01	0.43	0.19
Feed intake, % bwd^{-1}	2.15^{cd}	1.96^{e}	2.38^{b}	2.20^{bcd}	2.61^{a}	2.27^{bc}	2.05^{de}	0.05	0.001	0.01	0.01	0.51

Means ($n = 3$) in the same row with the same superscript are not significantly different ($P > 0.05$).
*Specific growth rate (SGR) = (final weight–initial wt)/duration of experiment (86 days).
†3 Feed conversion ratio (FCR) = g dry feed fed/g wet weight gain.

Table 3 Effect of diet on nutrient retention and carcass composition of rainbow trout

Source	Fish barley		Plant concentrate		Plant meals			Probability of $a > F$ value				
Density	Low	High	Low	High	Low	High	Commercial trout	SEM	Model	Source	Density	Source × density
PRE, %	36.0^{ab}	38.8^{ab}	35.5^{ab}	33.8^{ab}	33.1^{b}	34.1^{b}	37.8^{ab}	1.00	0.04	0.01	0.43	0.19
ERE, %	39.7^{ab}	40.8^{a}	36.3^{bc}	38.3^{ab}	31.8^{d}	35.0^{c}	37.6 ^{b}	1.48	0.01	0.01	0.01	0.51
Carcass composition												
Proteins, % dm	52.1^{ab}	54.6^{ab}	52.6^{ab}	48.5^{b}	51.8^{ab}	51.0^{ab}	53.0^{ab}	0.82	0.04	0.06	0.39	0.06
Lipid, % dm	37.1^{c}	38.2^{bc}	40.3^{abc}	43.2^{a}	41.6^{ab}	41.4^{ab}	37.0^{c}	0.77	0.07	0.01	0.20	0.98
Moisture, %	70.8^{a}	70.3^{ab}	69.5^{ab}	69.2^{b}	69.7^{ab}	69.2^{b}	71.0^{a}	0.39	0.01	0.01	0.02	0.07
Ash, % dm	1.75^{b}	1.74^{b}	1.82^{ab}	1.89^{ab}	1.97^{a}	1.83^{ab}	1.95^{a}	0.06	0.06	0.03	0.48	0.15

Means ($n = 3$) in the same row with the same superscript are not significantly different ($P > 0.05$).
PRE, protein retention efficiency; ERE, energy retention efficiency.

either as g fish $^{-1}$ or SGR, was greater for the fish fed the F–B diets (211.3 g fish $^{-1}$) than the fish either the PC diets (194.0 g fish $^{-1}$) or the PM diets (192.6 g fish $^{-1}$). The fish fed the commercial trout diets gained an average of 229.7 g fish $^{-1}$, and this was not different from the fish fed the HND F–B diet (224.0 g fish $^{-1}$).

Feed conversion ratios were good for fish fed any of the diets, and there were also significant effects of both protein source and nutrient density on FCR (Table 2). The fish fed the F–B diets had the best FCR (0.88) compared with 0.98 for the fish fed the PC diets and 1.05 for the fish fed the PM diets. Increasing the nutrient density of the diets significantly improved FCR from 1.02 for the fish fed the LND to 0.92 for the fish fed the HND diets. The interaction of the two dietary effects was not significant (0.12) (Table 2).

Apparent PRE was higher for trout fed the F–B diets (37.4%) than for trout fed the PM (34.6%) or PC diets (33.6%) (Table 3). Protein retention efficiency was not affected by the nutrient density of the diet. There was a consistent pattern of the effect of protein source on PRE and ERE. Trout fed the F–B diets had higher PRE and ERE values than fish fed either the PC or PM diets (Table 3).

The effect of diet on carcass composition was similar to the effect of diet on PRE and ERE (Table 3). There was a significant effect of protein source on carcass composition (Table 3). Fish fed the F–B diets had higher carcass protein and moisture and lower ash and fat than fish fed diets containing PC or PM.

Diet had a highly significant effect on ADC for organic matter, dry matter, protein lipid and energy, as well as on AAC for total amino acids and each of the ten essential amino acids (Table 4). The nutrient density of the diet affected the ADC for organic matter, dry matter and energy, but not protein, lipid or amino acids, with higher ADCs observed for fish fed the diets in HND series.

Plasma amino acids

There was an effect of dietary protein source on most of the plasma amino acid concentrations (Table 5). Plasma arginine concentrations were lower for fish fed the PM diets relative to fish fed the PC diets. Fish fed the F-B diets had plasma arginine concentrations intermediate to those groups. Dietary protein source also had a significant effect on plasma lysine concentration. Fish fed the F–B diet had higher plasma lysine concentrations than the PM fed fish and the PC treatment had intermediate lysine concentrations. Only one of the plasma concentrations of branched chain amino acids was affected by protein source. Plasma leucine concentration was higher for fish fed the PM diets to fish fed F–B or PC diets, which were equivalent. Increasing nutrient density results in increased plasma leucine concentrations. Of the other two branched chained amino acids, isoleucine concentrations were unaffected by dietary treatments while valine concentrations were higher in HND treatments compared with LND treatments with no protein source effects.

Plasma methionine concentrations were affected by dietary protein source but not nutrient density, with a significant interaction occurring. Within the HND treatments, fish fed the F-B diet had a higher plasma methionine concentration relative to fish fed the PC or PM diets, which were equivalent. Within the LND treatments, that fed the PM diet had the lowest methionine concentration. Plasma threonine con-

Table 4 Apparent digestibility (ADC) or availability (AAC) coefficients of dietary nutrients and energy for rainbow trout

	Fish barley		Plant concentrate		Plant meals		Probability of $a > F$ value				
ADC or AAC (%)	Low	High	Low	High	Low	High	SEM	Model	Source	Density	Source × density
Organic matter	77.6^{b}	85.1^{a}	68.5^{d}	73.7^{c}	72.3^{c}	73.8^{c}	0.88	0.01	0.01	0.01	0.01
Dry matter	74.9^{b}	80.9^{a}	64.6^{d}	69.3^{c}	68.6^{c}	68.9^{c}	0.88	0.01	0.01	0.01	0.04
Protein	91.0^{a}	91.2^{a}	85.8^{d}	86.7^{bc}	89.4^{b}	88.1^{cd}	0.78	0.01	0.01	0.92	0.01
Lipid	99.1^{a}	99.1^{a}	97.1^{b}	99.2^{a}	95.7^{c}	93.7^{d}	0.90	0.01	0.01	0.87	0.01
Energy	82.8^{b}	87.9^{a}	74.5^{d}	73.7^{d}	79.0^{c}	79.8^{c}	0.72	0.01	0.01	0.01	0.01
Total amino acids	95.1^{a}	95.2^{a}	88.7^{c}	90.3^{bc}	93.5^{a}	90.7^{b}	0.74	0.01	0.01	0.46	0.01
Arginine	97.2^{ab}	96.5^{b}	95.5^{c}	96.4^{b}	97.4^{a}	97.7^{a}	0.56	0.01	0.01	0.48	0.03
Histidine	94.3^{a}	95.0^{a}	90.3^{c}	92.1^{b}	93.8^{ab}	90.4^{c}	0.75	0.01	0.01	0.52	0.01
Isoleucine	95.9^{a}	96.4^{a}	88.0^{c}	89.9^{c}	92.4^{b}	88.7^{c}	0.55	0.01	0.01	0.39	0.01
Leucine	96.4^{a}	96.7^{a}	87.3^{c}	89.4^{b}	94.8^{a}	90.7^{b}	0.66	0.01	0.01	0.29	0.01
Lysine	95.6^{ab}	96.3^{a}	94.4^{c}	94.7^{bc}	93.1^{d}	92.8^{d}	0.58	0.01	0.01	0.34	0.25
Methionine	95.1^{a}	96.0^{a}	78.7^{c}	79.5^{c}	94.1^{ab}	91.4^{b}	0.90	0.01	0.01	0.66	0.13
Phenylalanine	96.1^{a}	96.0^{a}	89.9^{d}	91.7^{c}	95.1^{a}	93.2^{b}	0.58	0.01	0.01	0.91	0.01
Tyrosine	94.5^{a}	95.0^{a}	85.0^{c}	89.2^{b}	91.8^{a}	88.3^{a}	0.75	0.01	0.01	0.52	0.01
Threonine	96.8^{a}	96.2^{a}	87.3^{d}	91.5^{c}	95.2^{b}	95.0^{c}	0.59	0.01	0.01	0.03	0.01
Valine	94.7^{a}	95.7^{a}	86.6^{c}	88.4^{c}	91.0^{b}	88.1^{c}	0.70	0.01	0.01	0.93	0.01

Means ($n = 3$) in the same row with the same superscript are not significantly different ($P > 0.05$).

centrations were influenced by both nutrient density and protein source, and no interactions were observed. Fish fed the diets that contained fish meal (F–B) had elevated plasma threonine concentrations relative to the other protein sources and the fish consuming the high-density diets had elevated plasma threonine concentrations compared with the low-density dietary treatments. Plasma tryptophan concentrations were also affected by dietary protein source. Fish fed the PC and F–B diets had higher plasma tryptophan concentrations than fish fed the PM diets.

The plasma concentrations of aromatic amino acids phenylalanine and tyrosine were affected by dietary protein source. Only phenylalanine concentrations were significantly affected by nutrient density, and there was a significant interaction with protein source. Plasma phenylalanine and tyrosine concentrations were reduced in fish fed the F–B diets relative to fish fed diets containing other protein sources.

Histology

The kidneys appeared to be normal for fish fed F–B, PC, PM diets. Livers cell vacuolation, which is an indicator of cytoplasmic glycogen or fat storage, varied between mild to moderate for fish fed all diets. Very little

Table 5 Plasma amino acid concentrations of rainbow trout (nmol mL^{-1})

	Fish barley		Plant concentrate		Plant meals		Probability of $a > F$ value				
	Low	High	Low	High	Low	High	SEM	Model	Source	Density	Source × density
Arginine	308^{bc}	352^{ab}	361^{a}	383^{a}	277^{c}	269^{c}	16.2	0.01	0.01	0.17	0.30
Histidine	113^{b}	114^{b}	153^{a}	147^{ab}	137^{ab}	142^{ab}	9.8	0.08	0.01	0.98	0.88
Isoleucine	201	227	182	210	160	207	18.9	0.42	0.42	0.10	0.87
Leucine	359^{c}	403^{c}	351^{c}	383^{c}	546^{b}	713^{a}	31.6	0.01	0.01	0.02	0.17
Lysine	574^{ab}	678^{a}	563^{b}	542^{b}	529^{b}	486^{b}	38.2	0.04	0.02	0.66	0.12
Methionine	242^{bc}	329^{a}	318^{ab}	128^{d}	146^{d}	195^{cd}	23.9	0.01	0.01	0.42	0.01
Phenylalanine	192^{c}	193^{c}	241^{b}	223^{bc}	243^{b}	339^{a}	13.6	0.01	0.01	0.04	0.01
Tyrosine	114^{c}	123^{c}	177^{b}	185^{b}	199^{b}	247^{a}	14.4	0.01	0.01	0.08	0.31
Threonine	365^{b}	454^{a}	289^{bc}	308^{bc}	229^{c}	280^{bc}	25.5	0.01	0.01	0.04	0.49
Tryptophan	49^{ab}	52^{a}	57^{a}	52^{a}	36^{c}	37^{bc}	3.8	0.01	0.01	0.89	0.58
Valine	516^{ab}	617^{a}	535^{ab}	610^{a}	396^{b}	513^{ab}	43.0	0.09	0.07	0.04	0.92

Means ($n = 3$) in the same row with the same superscript are not significantly different ($P > 0.05$).

difference was noted in the ascending intestines of fish fed each of the diets. Absorptive vacuoles were present in the mucosal epithelium of the descending intestine of fish fed any of the diets. Occasionally, focal areas of mucosal epithelium lacking absorptive vacuoles were observed. While fusion of villi was apparent in fish fed each of the diets, in no case was it severe.

Discussion

Complete replacement of fish meal protein with plant protein without a reduction in growth has been the goal of many studies (Kaushik *et al.* 1995; Adelizi *et al.* 1998; Lee *et al.* 2002; Yamamoto *et al.* 2002). When analysed for main effects in the current trial, protein source did affect weight gain. The effect of protein source on weight gain was similar to that reported by Adelizi *et al.* (1998), with lower weight gain and higher FCRs for fish fed fish meal-free diets. The control feed in the studies conducted by Adelizi *et al.* (1998) was a commercial feed assumed to contain a high level of fish meal. Lee *et al.* (2002) observed the growth of trout fed a blend of animal proteins (blood meal, meat and bone meal, feather meal, poultry by-products meal and krill hydrolysate) to be less than the trout fed the 40% fish meal control diet. When the level of the animal protein mixture was reduced and cottonseed meal was added, fish growth increased to levels equivalent to the fish fed the control diet (Lee *et al.* 2002).

Comparison of treatment means in the current study, however, indicated that fish fed four fish meal-free diets had growth rates comparable to fish fed a fish meal-containing LND diet. This analysis is not as sensitive as the factorial analyses, but represents the type of comparison that would be made in a standard feeding study. The difference in weight gain within the LND series was only 8% between the fish fed F–B and PM and was not significantly different. Kaushik *et al.* (1995) similarly observed no difference in the growth rate of trout fed a soy protein concentrate diet as compared with trout fed a fish meal-based diet. Yamamoto *et al.* (2002) fed a diet that had the primary protein ingredients of meat and bone meal, soybean meal and corn gluten meal and observed a growth rate equivalent to trout fed a fish meal-based diet. This diet, however, was supplemented with L-isoleucine, threonine, tryptophan, valine, lysine and methionine. Isoleucine is currently too expensive for use in practical diets and needs to be supplied from intact protein sources.

Changes in plasma amino acid concentrations caused by changes in diet composition have been well documented (Yamamoto, Unuma & Akiyama 2000; Aoki, Akimoto & Watanabe 2001; Sunde, Kiessling, Higgs, Opstvedt, Venturini & Rungruangsak-Torrissen 2003). The significance of changes in plasma amino acid profiles is often less clear, but Sunde *et al.* (2003) determined that plasma amino acid profiles could be utilized to assess protein quality for Atlantic salmon. One necessary characteristic of this approach is that the differences caused by dietary protein quality have to be ranked relative to a control group within an experiment. The need for ranking is, in part, due to time-course post-prandial fluctuations in plasma amino acid concentrations. Ok, Bai, Park, Choi and Kim (2001) and Schuhmacher, Wax and Gropp (1997) noted that a peak in plasma amino acids occurs between 4 and 18 h post prandial in rainbow trout; differences between the two experiments may be partially attributed to water temperatures which were 17 °C and 10 °C respectively. It has also been well established that peaks in plasma amino acid concentrations occur more rapidly when crystalline amino acids are utilized to meet dietary amino acid requirements instead of intact protein (Ng, Hung & Herold 1996; Schuhmacher *et al.* 1997). In order to ensure equivalent comparisons between diets on plasma amino acid concentrations, several variables have to be constrained. In the current experiment, the effect of environmental temperature was controlled and the effect of post-prandial peaks due to feeding crystalline amino acids was minimized by feeding three times per day. The experimental design used in the present study allowed for differences in feed consumption among fish within a tank and is an uncontrolled source of variation, thus adding to the experimental error.

Yamamoto *et al.* (2000) determined that amino acid-imbalanced diets can also cause fluctuations in plasma amino acids. Plasma methionine was lower when an imbalanced amino acid mixture was fed to trout even though the methionine content of the diets was similar. In the current experiment, plasma methionine concentrations were influenced by protein source and a significant interaction occurred between protein source and energy density of the diet. In general, fish fed diets with fish meal protein had higher plasma methionine concentrations relative to fish consuming plant proteins. The addition of synthetic methionine to the diet resulted in elevated plasma methionine concentrations equivalent to the levels observed for trout fed the fish meal-containing diets.

In the current experiment, fish consuming HND diets had elevated plasma phenylalanine concentrations relative to LND diets. This finding is similar to that of Yamamoto *et al.* (2000) where high-fat diets increased the plasma levels of phenylalanine and tyrosine in trout. These authors postulated that elevated concentrations of plasma phenylalanine and tyrosine may be due to reduced catabolism of these amino acids because dietary energy supply was elevated. In the current experiment, it was difficult to differentiate the potential effects of elevated dietary energy from fat and the potential effects of elevated dietary protein as observed by Yokoyama and Nakazoe (1991) and Yamamoto *et al.* (2000). One point of interest was the lack of interactions between nutrient source and density on any plasma amino acid, except methionine and phenylalanine. The metabolic significance of the plasma phenylalanine observation as noted above is not clear and further research is needed to characterize the potential effects of increased dietary energy/lipid on circulating phenylalanine concentrations.

Increasing the nutrient density of the diet increased weight gain and ERE, decreased feed intake, FCR and had no effect on PRE. In agreement with the current study, Raven, Devlin and Higgs (2006) also reported an increase in weight gain and feed efficiency as the protein and energy content of the diet increased. In contrast, however, these investigators observed an increase in feed intake as the protein and energy content of the diet increased. The nutrient content of those diets were varied by increasing the inclusion rate of ingredients such as LT-anchovy meal, squid meal, krill meal and fish oil, and decreasing the inclusion rate of cellulose from 25% to zero. These changes may have increased the palatability of the diet as cellulose decreased, thus increasing feed intake. Nutrient density was increased in the current trial by increasing fish oil and protein concentrations at the expense of the lower protein ingredients wheat flour or barley meal.

Histological examination found the kidneys and livers to be normal for fish in all dietary treatments. Very little differences were noted in the ascending intestines of fish fed all the diets. Some fusion of intestinal villi was observed for fish fed any of the diets, but it was not severe in any treatments. Krogdahl, Bakke-McKellep and Baeverfjord (2003) observed changes in the lamina propria of the distal intestine of Atlantic salmon fed diets containing 15% soybean meal. Refstie *et al.* (2000) fed a diet containing 30% soybean meal and 32% fish meal to both Atlantic salmon and rainbow trout. Both species of fish showed morphological changes in the intestine, but the growth rate was only reduced for the Atlantic salmon fed the soybean-containing diet relative to fish fed the fish meal control diet. Contrary to these results, the lamina propria of rainbow trout fed the PM diet (LND series) did not show changes relative to trout fed a fish meal diet. The PM diet contained 19% soybean meal and no fish meal. The difference in results among the current study and those by Krogdahl *et al.* (2003) and Refstie *et al.* (2000) could be due to species differences, higher dietary levels or differences in the anti-nutrient concentrations from different soybean cultivars used in the studies.

Conclusion

Factorial analyses revealed that protein source does affect growth rate, with trout fed the fish meal-free diets growing about 10% slower than the trout fed diets with over 40% fish meal. It is apparent that the fish meal-free diets either lack specific nutrient/nutrients or contain some type of anti-nutrient that reduced growth rate relative to trout fed the high fish meal-containing diets. Elevated FCRs and reduced PRE and ERE were also observed for trout fed the fish meal-free diets. These differences in growth efficiency are important due to their effect on effluent management. The PC diets are currently 38% more expensive and the PM diets are 28% less expensive, than the F-B diet. The fish meal-free, plant meal-based diet (PM) will be a benchmark for future improvements in plant-based trout feeds. This study provides further evidence that the growth rates of trout fed a fish-meal-free diet, using conventional and concentrated plant protein ingredients, are close to but not equivalent to growth of trout fed fish meal-based feeds.

Acknowledgments

This research was jointly funded by USDA/ARS, CRIS project 106-5366-21310-002-00D, National Program 106 and the University of Idaho. We thank Mike Casten and Lorrie VanTassel for fish care, and Lucas Porter for assistance in feed manufacturing.

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture (USDA).

References

Adelizi P.D., Rosati R.R., Warner K., Wu Y.V., Muench T.R., White M.R. & Brown P.B. (1998) Evaluation of fish-meal free diets for rainbow trout, *Onchorynchus myskiss*. *Aquaculture Nutrition* **4**, 255–266.

Association of Official Analytical Chemists (AOAC) (1990) *Official Methods of Analysis*, 15th edn. Association of Official Analytical Chemists, Arlington, VA, USA 1298pp.

Aoki H., Akimoto A. & Watanabe T. (2001) Periodical changes of plasma free amino acid levels and feed digesta in yellowtail after feeding non-fishmeal diets with or without supplemental crystalline amino acids. *Fisheries Science* **67**, 614–618.

Arndt R.E., Hardy R.W., Sugiura S.H. & Dong F.M. (1999) Effects of heat treatment and substitution level on palatability and nutritional value of soy defatted flour for coho salmon, *Oncorhynchus kisutch*. *Aquaculture* **180**, 129–145.

Arnesen P., Brattås L.E., Olli J. & Krogdahl Å. (1990) Soybean carbohydrates appear to restrict the utilization of nutrients by Atlantic salmon (*Salmo salar* L.). The current status of fish nutrition in aquaculture.. In: *The Proceedings of the Third International Symposium on Feeding and Nutrition in Fish* (ed. by M Takeda & T Watanabe), pp. 273–280. Tokyo: Tokyo University of Fisheries.

Bairagi A., Gosh K.S., Sen S.K. & Ray A.K. (2004) Evaluation of the nutritive value of *Leucaena leucocephala* leaf meal, inoculated with fish intestinal bacteria *Bacillus subtillis* and *Bacillus circulans* in formulated diets for roho, *Labeo rohita* (Hamilton) fingerlings. *Aquaculture Research* **35**, 436–446.

Barrows F.T. & Hardy R.W. (2001) Nutrition and Feeding. In: *Fish Hatchery Management* (ed. by G. Wedemeyer), 2nd edn, pp. 483–558. John Wiley & Sons, New York, NY, USA.

Bureau D.P., Harris A.M. & Cho C.Y. (1998) The effects of purified alcohol extracts from soy products on feed intake and growth of Chinook salmon (*Onchorynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*) diets. *Aquaculture* **161**, 27–43.

Bureau D.P., Harris A.M., Bevan D.J., Simmons L.A., Azevedo P.A. & Cho C.Y. (2000) Feather meals and meat and bone meals from different origins as protein sources in rainbow trout (*Oncorhynchus mykiss*) diets. *Aquaculture* **181**, 281–291.

Cheng Z.J., Hardy R.W. & Huige N.J. (2004) Apparent digestibility coefficients of nutrients in brewer's and rendered animal by-products for rainbow trout (*Oncorhynchus mykiss* (Walbaum)). *Aquaculture Research* **35**, 1–9.

Cho C.Y. & Slinger S.J. (1979) Apparent digestibility measurement in feedstuffs for rainbow trout. In: *Proceedings of the World Symposium on Finfish Nutrition and Fishfeed technology*, Hamburg, Germany, Vol. II, pp. 239–247.

Cho C.Y., Bayley H.S. & Slinger S.J. (1974) Partial replacement of herring meal with soybean meal and other changes inna diet for rainbow trout (*Salmo gairdneri*). *Journal of Fisheries Research Board of Canada* **31**, 1523–1528.

Dabrowska H. & Wojno T. (1977) Studies on the utilization by rainbow trout (*Salmo Gairdneri* Rich.) of feed mixtures containing soya bean meal and an addition of amino acids. *Aquaculture* **10**, 297–310.

Drew M.D., Racz V.J., Gauthier R. & Thiessen D.L. (2005) Effect of adding protease to coextruded flax: pea or canola: pea products on nutrient digestibility and growth performance of rainbow trout (*Oncorhynchus mykiss*). *Animal Feed Science and Technology* **119**, 117–128.

Erturk M.M. & Sevgili H. (2003) Effects of replacement of fish meal with poultry by-product meals on apparent digestibility, body composition and protein efficiency ratio in a practical diets for rainbow trout (*Oncorhynchus mykiss*). *Asian-Australian Journal of Animal Science* **16**, 1355–1359.

Encarnacao P., de Lange C., Rodehutscord M., Hoehler D., Bureau W. & Bureau D.P. (2004) Diet digestible energy content affects lysine utilization, but not dietary lysine requirements of rainbow trout (*Oncorhynchus mykiss*) for maximum growth. *Aquaculture* **235**, 569–586.

Fleming J., Taylor T., Miller C. & Woodward C. (1992) *Analysis of Complex Mixtures of Amino Acids Using the HP 1050 Modular HPLC. Application Note 228-212, Publication No. 5091-5615E.*. Agilent Technologies, Paulo Alto, CA, USA.

Fowler L.G. (1980) Substitution of soybean and cottonseed products for fish meal in diets fed to chinook and coho salmon. *The Progressive Fish-Culturist* **42**, 87–91.

Glencross B., Evans D., Dods K., McCafferty P., Hawkins W., Maas R. & Sipsas S. (2005) Evaluation of the digestible value of lupin and soybean protein concentrates and isolates when fed to rainbow trout, *Oncorhynchus mykiss*, using either stripping or settlement faecal collection methods. *Aquaculture* **245**, 211–220.

Glencross B., Hawkins W., Evans D., Rutherford N., Dods K., Maas R., McCafferty P. & Sipsas S. (2006) Evaluation of the nutritional value of prototype lupin protein concentrates when fed to rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **251**, 66–77.

Gomes E.F., Rema P. & Kaushik S.J. (1995) Replacement of fish meal by plant protein in the diet of rainbow trout (*Oncorhynchus mykiss*): digestibility and growth performance. *Aquaculture* **130**, 177–186.

Higgs D.A., Markert J.R., Macquarrie D.W., McBrie J.R., Dosanjh B.S., Nichols C. & Hoskins G. (1978) Development of practical dry diets for coho salmon, *Onchorynchus kisutch*, using poultry-by-product meal, feather meal, soybean meal and rapeseed meal as major protein sources. In: *Proceedings of World Symposium on Finfish Nutrition and Fishfeed Technology*, June 20–23, Vol. II, pp. 191–218. Hamburg, Germany.

Kaushik S.J., Cravedi J.P., Lalles J.P., Sumpter J., Fauconneau B. & Laroche M. (1995) Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic effects, cholesterolemia and flesh quality in rainbow trout. *Aquaculture* **133**, 257–274.

Ketola H.G. & Harland B.F. (1993) Influence of phosphorus in rainbow trout diets on phosphorus discharges in effluent

water. *Transactions of the American Fisheries Society* **122**, 1120–1126.

Krogdahl A., Berg-Lea T. & Olli J.J. (1994) Soybean proteinase inhibitors affect intestinal trypsin activities and amino acid digestibilities in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physioogyl* **107A**, 215–219.

Krogdahl A., Bakke-McKellep A.M. & Baeverfjord G. (2003) Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). *Aquaculture Nutrition* **9**, 361–371.

Lee K.J., Dabrowski K., Blom J.H., Bai S.C. & Stromberg P.C. (2002) A mixture of cottonseed meal, soybean meal and animal byproduct mixture as a fish meal substitute: growth and tissue gossypol enantiomer in juvenile rainbow trout (*Oncorhynchus mykiss*). *Journal of Animal Physiology and Animal Nutrition* **86**, 201–213.

Letang C., Samson M.F., Lasserre T.M., Chaurand M. & Abecassis J. (2001) Production of starch with very low protein content from soft and hard wheat flours by jet milling and air-classification. *Cereal Chemistry* **79**, 535–543.

Medale F., Brauge C., Vallee F. & Kaushik S.J. (1995) Effects of dietary protein/energy ratio, ration size, dietary energy source, and water temperature on nitrogen excretion in rainbow trout. *Water Science and Technology* **31**, 185–194.

Mukhopadhyay N. & Ray A.K. (1999) Effect of fermentation on the nutritive value of sesame seed meal in the diets for rohu, *Labeo rohita* (Hamilton), fingerlings. *Aquaculture Nutrition* **5**, 229–236.

National Research Council (NRC) (1993) *Nutrient Requirements of Fish*. National Academy Press, Washington, DC.

Ng W., Lim H., Lim S. & Ibrahim C. (2002) Nutritive value of palm kernel meal pretreated with enzyme or fermented with *Trichoderma kongii* (Oudemans) as a dietary ingredient for red hybrid tilapia (*Oreochromis* sp.). *Aquaculture Research* **33**, 1199–1207.

Ng W.K., Hung S.S.O. & Herold M.A. (1996) Poor utilization of dietary free amino acids by white sturgeon. *Fish Physiology and Biochemistry* **15**, 131–142.

Ok I.-H., Bai S.C., Park G.-J., Choi S.-M. & Kim K.-W. (2001) The patterns of free amino aicds after force-feeding in rainbow trout *Oncorhynchus mykiss* (Walbaum) with and without dorsal aorta cannulation. *Aquaculture Research* **32**(suppl 1), 70–75.

Olli J., Krogdahl A. & Berg-Lea T. (1989) Effects of soybean trypsin inhibitor activity on nutrient digestibility in salmonids fed practical diets containing various soybean meals. In: *Proceedings of Third International Symposium on Feeding and Nutrition in Fish*, Vol. 89 (ed. by M. Takeda & T. Watanabe), pp. 263–271. Japan Translation Center, Tokyo, Japan.

Rasmussen R.S., Ostenfeld T.H. & McLean E. (2000) Growth and feed utilization of rainbow trout subjected to changes in feed lipid concentrations. *Aquaculture International* **8**, 531–542.

Raven P.A., Devlin R.H. & Higgs D.A. (2006) Influence of dietary digestible energy content on growth, protein and energy utilization and body composition of growth hormone transgenic and non-transgenic coho salmon (*Oncorhynchus kisutch*). *Aquaculture* **254**, 730–747.

Refstie S., Korsoen O.J., Storebakken T., Baeverfjord G., Lein I. & Roem A.J. (2000) Differing nutritional responses to dietary soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Aquaculture* **190**, 49–63.

Refstie S., Storebakken T., Baeverfjord G. & Roem A.J. (2001) Long-term protein and lipid growth of Atlantic salmon (*Salmo salar*) fed diets with partial replacement of fish meal by soy protein products at medium or high lipid level. *Aquaculture* **193**, 91–106.

Refstie S., Sahlstrom S., Brathen E., Baeverfjord G. & Krogedal P. (2005) Lactic acid fermentation eliminates indigestible carbohydrates and anti-nutritional factors in soybean meal for Atlantic salmon (*Salmo salar*). *Aquaculture* **246**, 331–345.

Reinitz G. (1980) Soybean meal as a substitute for herring meal in practical diets for rainbow trout. *The Progressive Fish-Culturist* **42**, 103–106.

SAS Institute (1988) *SAS Procedures Guide 6.0*, 3rd edn. SAS Institute, Cary, NC, USA 441pp.

Schuhmacher A., Wax C. & Gropp J.M. (1997) Plasma amino acids in rainbow trout (*Oncorhynchus mykiss*) fed intact protein or a crystalline amino acid diet. *Aquaculture* **151**, 15–28.

Sheehan D. & Hrapchek B. (1983) *Theory and Practice of Histotechnology*, 2nd edn. Battlelle Press, Columbus, OH, USA.

Skrede G., Storebakken T., Skrede A., Sahlstrom S., Sorensen M., Shearer K.D. & Slinde E. (2002) Lactic acid fermentation of wheat and barley whole meal flours improves digestibility of nutrients and energy in Atlantic salmon (*Salmo salar* L.) diets. *Aquaculture* **210**, 305–321.

Sokal R.R. & Rohlf F.J. (1981) *Biometry*, 2nd edn. W.H. Freeman and Company, New York, USA 857pp.

Stone D.A.J., Hardy R.W., Barrows F.T. & Cheng Z.J. (2005) Effects of extrusion on nutritional value of diets containing corn gluten meal and corn distiller's dried grain for rainbow trout, *Oncorhynchus mykiss*. *Journal of Applied Aquaculture* **17**, 1–20.

Sunde J., Kiessling A., Higgs D., Opstvedt J., Venturini G. & Rungruangsak-Torrissen K. (2003) Evaluation of feed protein quality by measuring plasma free amino acids in Atlantic salmon (*Salmo salar* L.) after dorsal aorta cannulation. *Aquaculture Nutrition* **9**, 351–360.

Vielma J., Makinen T., Ekholm P. & Koskela J. (2000) Influence of dietary soy and phytase levels on performance and body composition of large rainbow trout (*Oncorhynchus mykiss*) and algal availability of phosphorus load. *Aquaculture* **183**, 349–362.

Weatherup R.N., McCracken K.J., Foy R., Rice D., McKendry J., Mairs R.J. & Hoey R. (1997) The effects off dietary fat on performance and body composition of farmed rainbow trout (*Oncoryhnchus mykiss*). *Aquaculture* **151**, 173–184.

Wu Y.V. & Stringfellow A.C. (1973) Protein concentrates from oat flours by air classification of normal and high-protein varieties. *Cereal Chemistry* **50**, 489–496.

Wu Y.V. & Stringfellow A.C. (1992) Air classification of flours from wheat of varying hardness: protein shifts. *Cereal Chemistry* **69**, 188–191.

Yamamoto T., Unuma T. & Akiyama T. (2000) The influence of dietary protein and fat levels on tissue free amino acid levels of fingerling rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **182**, 353–372.

Yamamoto T., Shima T., Furuita H. & Suzuki N. (2002) Influence of feeding diets with and without fish meal by hand and by self-feeders on feed intake, growth and nutrient utilization of juvenile rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **214**, 289–305.

Yigit M., Yardim O. & Koshio S. (2002) The protein sparing effects of high lipid levels in diets for rainbow trout (*Oncoryhnchus mykiss*, W. 1972) with special reference to reduction of total nitrogen excretion. *The Israeli Journal of Aquaculture–Bamidgeh* **54**, 79–88.

Yokoyama M. & Nakazoe J. (1991) Effects of dietary protein levels on free amino acid and glutathione contents in the tissues of rainbow trout. *Comparative Biochemistry and Physiology* **99A**, 203–220.

